

Stability and phenotypic adaptability by AMMI analysis in cowpea beans (*Vigna unguiculata* (L.) Walp.)¹

Estabilidade e fenotípica adaptabilidade por análise AMMI em feijão caupi (*Vigna unguiculata* (L.) Walp.)

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ABSTRACT- Cowpea beans (*Vigna unguiculata* (L.) Walp), is the most important legume in the Colombian Caribbean and is cultivated mainly by family-economy farmers under variable environmental conditions. This demands the identification of cultivars with greater adaptation and stability, since genotype - environment interaction (GEI) hinders the process of selection and recommendation of cultivars that, because of their genetic potential, exceed the current yield (600 kg ha⁻¹). The objective of this study was to evaluate the adaptability and stability of 10 cowpea bean genotypes of semi-prostrate habit in eight environments of the Colombian Caribbean, during the years 2017B and 2018A; using the randomized complete block design with four repetitions. The analysis was performed using the additive main effects and multiplicative interaction (AMMI) model. The results showed highly significant differences at the level of environments, genotypes and GEI. The first three axes of the interaction were significant, selecting the first two for accumulating 81.84% of the sum of squares of the genotype by environment interaction. Genotype 2 showed the greatest adaptability and stability, but its performance was slightly lower than the average; genotypes 6 and 10 presented the highest grain yields, but were influenced by the environments, with the best response in favorable environments. The best environments corresponded to VN8A and SA7B.

Key words: Genotype-environment interaction, Principal component analysis, Semi-prostrate habit, Environments, Genotypes.

RESUMO- O feijão caupi (*Vigna unguiculata* (L.) Walp), é a leguminosa mais importante do Caribe colombiano e é cultivado principalmente por agricultores familiares em condições ambientais variáveis. Isso exige a identificação de cultivares com maior adaptação e estabilidade, visto que a interação genótipo - ambiente (IGA) dificulta o processo de seleção e recomendação de cultivares que, devido ao seu potencial genético, permitem superar a produtividade atual (600 kg ha⁻¹). O objetivo deste estudo foi avaliar a adaptabilidade e estabilidade de 10 genótipos de feijão-caupi de hábito semipós-cultivado em oito ambientes do Caribe colombiano, durante os anos 2017B e 2018A; usando o delineamento de blocos completos casualizados com quatro repetições. A análise foi realizada utilizando o modelo de efeitos principais aditivos e interação multiplicativa (AMMI). Os resultados mostraram diferenças altamente significativas ao nível de ambientes, genótipos e IGA. Os três primeiros eixos da interação foram significativos, selecionando os dois primeiros por acumular 81,84% da soma dos quadrados do genótipo por interação com o ambiente. O genótipo 2 apresentou maior adaptabilidade e estabilidade, mas seu desempenho foi ligeiramente inferior à média; os genótipos 6 e 10 apresentaram os maiores rendimentos de grãos, mas foram influenciados pelos ambientes, com melhor resposta em ambientes favoráveis. Os melhores ambientes corresponderam a VN8A e SA7B.

Palavras-chave: Interação genótipo-ambiente Análise de componentes principais. Hábito semiprostrado. Ambientes. Genótipos.

DOI: 10.5935/1806-6690.20210032

Editor-in-Chief: Prof. Alek Sandro Dutra - alekdutra@ufc.br

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Received for publication on 13/04/2020; approved on 10/08/2020

¹This article is a product of the Research Project Funded by Colciencias, Universidad de Córdoba and Fundación Canal del Dique de Compas

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INTRODUCTION

Cowpea beans are the most important legume in the Colombian Caribbean, cultivated by small producers with Creole cultivars, which show low yield (600 kg ha^{-1}) due to the use of non-certified seeds and susceptibility to abiotic factors such as drought, temperature, and biotics such as bacteria, weeds, insects, and fungi (PATRIYAWATY; RACHAPUTI; GEORGE, 2018; SINDHU *et al.*, 2019).

Among the species cultivated in semi-arid tropical and subtropical areas, cowpea beans stand out (KIRIGIA *et al.*, 2018), with phenotypic plasticity which allows it to adapt to drought and high temperatures, to guarantee grain production, although not all cultivars respond in the same way (ALVES-BARROS *et al.*, 2013; EL-SHAIENY *et al.*, 2015). It is sensitive to drought stress, especially in the flowering period (GULL; SOFI; ARA, 2019), affecting yield components (MEENA; RAM-KRISHNA; SINGH, 2015), which could be aggravated by the effects of climate change and climate variability on agriculture (IPCC, 2014). In this sense, studies carried out by Singh *et al.* (2010), in cowpea beans, showed that the interaction between CO_2 , temperature and ultra violet rays differentially affect cultivars, especially in the reproductive stage, with reduction in the number of fruiting branches, pollen viability and, consequently, the yield, which depends on the time of stress (SAGE *et al.*, 2015).

Yield is a characteristic of polygenic nature affected by the environment, which makes genetic improvement difficult (WALLE *et al.*, 2019); in this sense, cowpea beans are cultivated in Colombia, in areas that show a wide variety of environmental conditions associated with the hydric state of the soil, fertility, solar radiation, temperature, types of soil and production technologies; that cause differential phenotypic values of the genotypes according to the environmental supply, a phenomenon called genotype-by-environment interaction (OLIVEIRA *et al.*, 2017; SÁNCHEZ-RAMÍREZ; MENDOZA-CASTILLO; MENDOZA-MENDOZA, 2016).

For genetic improvement programs in their final phase, the evaluation of promising genotypes under heterogeneous environmental conditions of the producing areas (localities and years) where the crops are established is of great relevance. With this type of evaluation, the phenotypic expression of the plant in various environmental conditions is better understood, since the presence of genotype-by-environment interaction makes the selection process difficult due to the sensitivity of the plants to the growing conditions (VAEZI *et al.*, 2019). Analysis of the interaction of genotypes with environments is effective in characterizing the response of genotypes in the various environments and allows the selection of those with wide adaptation and stability (LIGARRETO M.; CASTRO H.;

CHÁVES C., 2015; TOSQUY-VALLE *et al.*, 2019).

Various methods have been carried out in cowpea in order to release cultivars, among them, the AMMI (additive main effects and multiplicative interaction) model, which is multivariate and appropriate for analyzing two-dimensional matrices, such as those containing genotype and environmental data (EL-SHAIENY *et al.*, 2015; MONDO; KIMANI; NARLA, 2019; TOSQUY-VALLE *et al.*, 2019). The AMMI statistics presented in biplots can be used to provide insightful interpretation of data from large and complex experiments (EBDON; GAUCH JR, 2002).

The objective of this study was to evaluate the adaptability and stability of cowpea bean genotypes of semi-prostrate habit, under environmental conditions of the Caribbean region of Colombia.

MATERIAL AND METHODS

The grain yield of nine advanced lines of the genetic breeding program of the University of Córdoba, together with the commercial control Caupicor 50, was evaluated in eight environments of the Caribbean region of Colombia in 2017-2018, characterized by being contrasting in their fertility, precipitation and temperatures (Table 1). These materials are characterized by their semi-prostrate growth habit and cream-colored seeds with black hilum. The agronomic evaluation of the genotypes was carried out under an experimental design in randomized complete blocks with 10 treatments and four repetitions per environment. Each experimental unit consisted of six rows of five meters long with 0,80 m between rows and 0,40 m between plants for a density of 31,250 plants per hectare. The two central rows were considered as a useful plot and, to avoid the edge effect, two additional rows of the commercial cultivar were placed at the ends. The grain yield data, in kg/ha, were corrected for 13% humidity and analyzed using statistical models for analysis of variance (MARTÍNEZ BECERRA; MARTÍNEZ RUEDA, 1997).

The Caribbean region of Colombia has a typical tropical savanna climate subtype Aw, according to the Köppen classification, with a temperature variation between 24 and 28 °C and annual rainfall between 800 and 1800 mm. This region has soils that differ from one area to another with fertility that varies from high to very low, depending on precipitation and the influence or not of rivers and tributaries. In general, for agricultural activities, two large sub-regions with specific variations in precipitation and soils have been identified: The Dry Caribbean, with higher temperatures and less precipitation, and the Humid Caribbean, with lower temperatures, greater precipitation and soil fertility.

The statistical model for the combined analysis of the localities is:

Table 1 - Cowpea bean genotypes evaluated in eight environments of the Colombian Caribbean

Genotype	Origin†	Environments*	Subregion
LC-029-016	IT86 x LCPM-35	Leticia – Bolívar: LE8A	Humid C.
LC-002-016	IT86 x LCPM-35	Mahates-Bolívar: MA7B	Humid C.
LC-036-016	IT86 x LCPM-35	Montería – Córdoba: MO7B	Humid C.
LC-009-021	IT86 x LCPM-35	Polonuevo-Atlántico: PN7B	Dry C.
LC-029-016	IT86 x LCPM-35	Polonuevo – Atlántico: PN8A	Dry C.
L-019	Criollo Córdoba	Sampué-Sucre: SA7B	Humid C.
LC-006-016	IT86 x LCPM-35	Sampué-Sucre: SA8A	Humid C.
LC-005-016	IT86 x LCPM-35	Villanueva – Guajira: VN8A	Dry C.
LC-014-016	IT86 x LCPM-35		
Caupicor 50	Commercial Cultivar		

†: Individual selection method; *: Municipalities of the Colombian Caribbean, second half of 2017 and first half of 2018. Humid Caribbean: Humid C.; Dry Caribbean: Dry C

$$Y_{ijk} = \mu + \pi_i + \rho_{ij} + \tau_k + (\pi\tau)_{ik} + \varepsilon_{ijk} \quad (1)$$

$$i = 1, 2, \dots, l; j = 1, 2, \dots, r; k = 1, 2, \dots, t$$

Where: Y_{ijk} : response or characteristic under study; μ : general mean; π_i : effect of the i -th locality; ρ_{ij} : effect of the j -th block within the i -th location; τ_k : effect of the k -th treatment; $(\pi\tau)_{ik}$: effect of the interaction of the i -th location and the k -th treatment; ε_{ijk} : is the experimental error in the i -th location, in the j -th block under the k -th treatment. Both the analysis of variance by environment and the combined one of the localities were processed with SAS software, version 9.4.

The effect of the genotype-environment interaction (GEI) was analyzed by means of the AMMI (additive main effects and multiplicative interaction) model. This model combines the analysis of variance for the principal effects of genotypes and environments with the analysis of principal components for GEI. The AMMI model considers that the effects of genotypes and environments are additive and linear, while the GEI has multiplicative effects that can be studied by principal component analysis (EBDON; GAUCH JR, 2002). A detailed description of the fixed effects model for AMMI (ZOBEL; WRIGHT; GAUCH JR, 1988) has the following structure:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum_{n=1}^N \lambda_n \zeta_{gn} \eta_{en} + \varepsilon_{ger} \quad (2)$$

Where: Y_{ger} : performance of genotype g in environment e for repetition r ; μ : general mean; α_g : effect of genotype g : mean deviation of the genotype (mean minus the general mean); β_e : effect of the environment; e : mean deviation of the environment; N : number of axes in the principal component analysis (PCA) retained in the model; λ_n : square root of the eigenvalue for the n -axis of the PCA; ζ_{gn} and η_{en} : PCA scores for their own axes ε_{ger} : error of model.

The eigenvectors (scores) in the PCA for genotypes and environments are dimensionless, while the units for the eigenvalues, λ , have the same units as the response variable (EBDON; GAUCH JR, 2002; ZOBEL, 1988). The software GEA-R (Genotype \times Environment Analysis with R for Windows) Version 4.1 was used for AMMI analysis; in the International Maize and Wheat Improvement Center CIMMYT (PACHECO *et al.*, 2017).

The objective of this study was to evaluate the adaptability and stability of cowpea bean genotypes of semi-prostrate habit, under the environmental conditions of the Caribbean region of Colombia.

RESULT AND DISCUSSION

The combined analysis of variance estimated significant differences between genotypes, between environments and in the genotype \times environment interaction (Table 2), indicates that the grain yield of cowpea varies according to the environments, and is consistent with the results of Alves-Barros *et al.* (2013), and El-Shaieny *et al.* (2015). The existence of this significant interaction leads to studying the stability and adaptability of the genotypes in the various environments.

Table 3 shows the results of the estimates of the mean squares for the main additive effects and genotype - environment interaction (GEI) for the grain yield between environments and genotypes, with statistical evidence of the variability between environments and between genotypes. The existence of GEI is based on the genetic differences between genotypes and environmental factors such as fertility, precipitation and temperature between the

Table 2- Analysis of variance for grain yield of 10 cowpea bean genotypes in eight environments of the Caribbean Region of Colombia 2017-2018

Sources of variation	Degrees of freedom	Mean squares	Pr >F
Environment (E)	7	8563295.5**	<0.0001
Block (Environments)	24	101754.2**	0.0026
Genotype (G)	9	1115131.3**	<0.0001
G x E	63	214723.14**	<0.0001
Error	216	47988.2	
Mean	1,134.8		
CV (%)	19.30		
R2	0.89		

humid and the dry Caribbean (GULL; SOFI; ARA, 2019; SINDHU *et al.*, 2019; SINGH *et al.*, 2010), which justifies carrying out a study of genotypes to know the magnitudes of their interaction with environments (OLIVEIRA *et al.*, 2017; SÁNCHEZ-RAMÍREZ; MENDOZA-CASTILLO; MENDOZA-MENDOZA, 2016), since it affects the correlation and reliability between phenotypic and genotypic values and makes it difficult to select and recommend superior cultivars (LIGARRETO M.; CASTRO H.; CHÁVES C., 2015; VAEZI *et al.*, 2019).

In the breakdown of total variation in treatments, it was observed that environmental effects were responsible for the greatest variation with a value of 71.78%, followed by GEI with 16.19% and finally, the contribution of genetic differences in cultivars with 12.01%. This is consistent with Mondo, Kimani and Narla (2019) and Vaezi *et al.* (2019), who argued that this occurs due to the strong interaction between years and localities, in which the stresses caused by abiotic factors, especially in the dry Caribbean localities, mainly affect the number of pods per plant and number of seeds per pod (WALLE *et al.*, 2019). In addition, high temperatures in the reproductive stage affect pollen production and viability (PATRIYAWATY; RACHAPUTI; GEORGE, 2018), since temperatures are higher in the dry Caribbean relative to the humid Caribbean.

According to the Gollob test (1968), in the AMMI analysis, the mean squares of the GEI show that the first two components PC1 and PC2 account for 81.84% of the variation (Sum of Square of the GEI interaction) and 44.44% of the degrees of freedom, while the first three PC1, PC2 and PC3, accounted for 93.34% and 61.90%, respectively. The rest of the principal components were not significant (Table 3). The explanation of GEI, in a little more than 80% of the variation, with the first two components is satisfactory to understand the interaction between genotypes and environments (LIGARRETO M.; CASTRO H.; CHÁVES C., 2015; TOSQUY-VALLE *et al.*, 2019).

The GEI residual made up of PC4 to PC7 (Table 3) represents a mean square (MS) of 37,552.68 with 24 degrees of freedom (df) not significant with an F of only 0.70. Furthermore, if a GEI residual made up of PC3 to PC7 is considered, it will still be insignificant, since the resulting MS is 70,198.06 with 35 df for a F-value of 1.32. The partitioning of the sum of squares (SS) by the AMMI model was quite effective, given that the mean square of the first axis of the PCA is more than 14 times the MS of the estimated residual of the interaction from PC4 to PC7, and 13 times the estimate from PC3 to PC7. On the other hand, the AMMI model without the error term contains 98.92% of the SS and the residual of the interaction was 1.08% when the grouping to estimate the GEI residual was made from the fourth principal component to the last. When the grouping was done from the third principal component, 97.06 and 2.94% were obtained, respectively.

In either of the two cases, the GEI residual indicates that the model to be interpreted is the AMMI2 and the variation present in the rest of the principal components is negligible and makes noise, which may reduce the efficiency of interpretation of the stability of the genotypes and the environment in the graphic analysis according to what was reported by Mondo, Kimani and Narla (2019) in common beans; Sánchez-Ramírez, Mendoza-Castillo and Mendoza-Mendoza (2016) in corn and Tadesse *et al.* (2017) in bean.

For the analysis of stability, the first step consisted in estimating the existence of significant differences between the yields of the genotypes in the evaluated environments. Subsequently, the sources of variation were broken down (Table 3) to estimate the effects of GEI on principal components to continue with the study of adaptability and stability.

Figure 1 presents the biplot in which the abscissa shows the principal effects (means of the genotypes and environments) and the ordinate the first axis of the PCA. Therefore, this AMMI1 has 59.97% of the total

Table 3. Mean squares of the additive main effects and multiplicative interaction through the Analysis of variance and Gollob test of the AMMI Model of 10 cowpea bean genotypes evaluated in eight environments of the Colombian Caribbean 2017-2018

SV	DF	MS	% SS	% SS _{GxA}	Prob. F
Environment (E)	7	8563295.50**	71.78		0.00000
Genotype (G)	9	1115131.34**	12.02		0.00000
G x E	63	214723.14**	16.20		0.00000
PC1	15	540815.30	59.97**	59.97	0.00000
PC2	13	227568.95	21.87**	81.84	0.00000
PC3	11	141424.35	11.50**	93.34	0.00322
PC4	9	42390.73	2.82	96.16	0.62169
PC5	7	48247.18	2.50	98.65	0.50378
PC6	5	32514.95	1.20	99.86	0.69278
PC7	3	6480.93	0.14	100.00	0.94739
PC8	0	0.00	0.00	100.00	1.00000
Residual	240	53364.79			

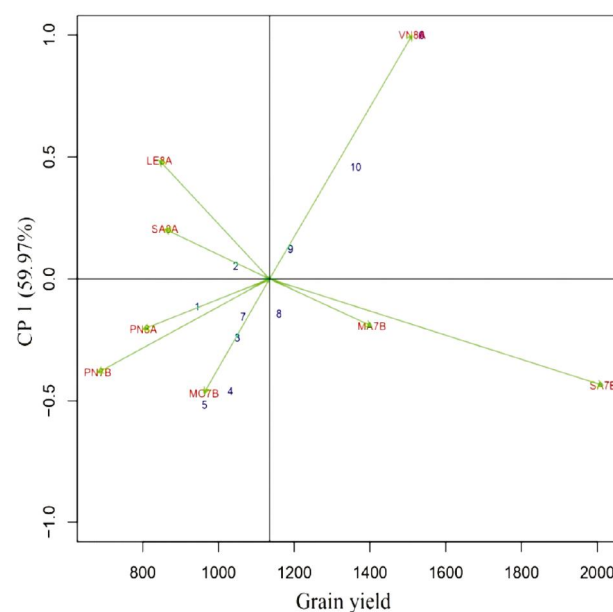
SV = source of variation; DF = degrees of freedom; MS = mean squares; % SS = percentage of the total sum of squares; % SS_{GxA} = percentage of the sum of squares of the genotype - environment interaction; G x E = genotype by environment interaction; PC1, PC2, ..., PC8 = principal component 1, 2, ..., 8, respectively; ** = significant with $P \leq 0.01$

sum of squares (SS) of the GEI and 93,52% of the SS of the reduced AMMI model, without the error term. Furthermore, the GEI is shown in a uni-dimensional way (GAUCH JR; PIEPHO; ANNICCHIARICO, 2008). Given the dispersion of genotypes and environments, it was observed that GEI is appreciable, which is due to the genetic complexity of performance (MONDO; KIMANI; NARLA, 2019; SÁNCHEZ-RAMÍREZ; MENDOZA-CASTILLO; MENDOZA-MENDOZA, 2016), as well as environmental divergence in the Caribbean region of Colombia. Hence the differential response of genotypes in different environments, is due to the magnitude of their contribution to genotype - environment interaction (EL-SHAIENY *et al.*, 2015).

In general, genotypes 1, 2, 7, 8 and 9 have small effects on interaction, that is, they are little affected by environments, while genotypes 3, 4, 5, 6 and 10 have high effects on interaction with environments, with instability in yield.

Genotypes 8 and 9 showed above average yield and were slightly affected by environment, in contrast genotypes 6 with specific adaptability for VN8A and 10 showed the highest yields, but were significantly affected by environments. These two genotypes responded positively to the extent that the environment was favorable. Similar results were reported by Ligarreto M., Castro H. and Cháves C. (2015) for common beans, which could be associated with the influence of yield components such as the number of pods per plant, number of seeds per pod and weight of 100 seeds (MEENA; RAMKRISHNA; SINGH, 2015; MENDONÇA *et al.*, 2018). On

Figure 1. Biplot analysis for grain yield (kg ha⁻¹) of 10 cowpea cultivars evaluated in eight environments of the Colombian Caribbean. 1= LC-029-016; 2=LC-002-016; 3=LC-036-016; 4=LC-009-016; 5=LC-021-016; 6=L-019; 7=LC-006-016; 8=LC-005-016; 9=LC-014-016; 10=Caupicor 50; 1=LE8A; 2=MA7B; 3=MO7B; 4=PN7B; 5=PN8A 2018; 6=SA7B; 7=SA8A; 8=VN8A



the other hand, genotypes 4 and 5 had a yield below the general mean (Figure 1) and are also affected by different environments.

At the level of environments, MA7B, VN8A and SA7B, turned out to be the most favorable, as the 10 genotypes showed average values above 1,400 kg ha⁻¹, while PN7B was the least favorable with an average of 700 kg ha⁻¹ (Table 4).

The AMMI2 analysis combined the first two principal components of the GEI, which added up to 81.84% of the SS of the GEI and 97.06% of the SS of the reduced AMMI model, without the error term (Figure 2). In the biplot of the AMMI2 analysis, the SA8A environment is the closest to the origin and contributed the most to the phenotypic stability of the genotypes. However, it was in this environment that the second lowest yield was recorded, 850 kg ha⁻¹. On the other hand, the VN8A, SA7A and LE8A environments, those furthest from the origin of Figure 1, contributed more to the GEI, that is, to the instability of the genotypes (Table 4); the results were concordant with those reported in common beans by Mondo, Kimani and Narla (2019).

Genotypes close to the environments were positively associated: genotype 2 showed adaptation to all environments, it showed the greatest phenotypic stability, although its average yield was barely 1,045 kg/ha; genotype 3 adapted to 50% of the environments: PN7B, PN8A, MA7B and MO7A, with an average yield of 1,050 kg ha⁻¹; genotypes 1 and 8, with yields of 944 and 1,159 kg ha⁻¹, showed adaptation to the SA7B environment; genotype 5 showed very good adaptation to the PN7B environment and its average yield was 963 kg ha⁻¹; genotype 10 adapted to the VN8A and SA8A environments and its average yield was 1,362 kg ha⁻¹; genotype 4 showed adaptation to PN8A and PN7B environments; 7 to MA7B; genotypes 6 and 10 were the most unstable, although the first registered the highest yield, 1,536 kg ha⁻¹ (Table 4).

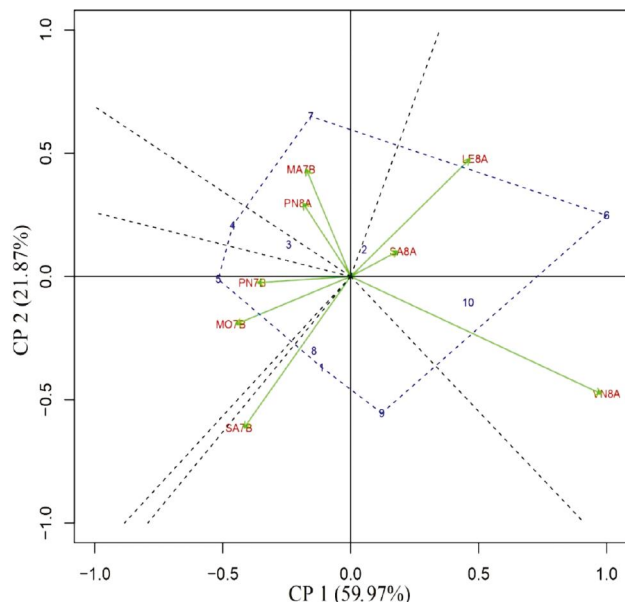
For commercial recommendation purposes, genotypes must present both agronomic characteristics and excellent yields, such is the case of genotype 6, which outperformed the rest, including the commercial control genotype 10.

Table 4 - Averages of genotypes and environments for grain yield (kg ha⁻¹) of cowpea by the additive main effects and multiplicative interaction (AMMI) model, plus scores of the first three axes of the principal component analysis, for 10 semi-prostrate cultivars in eight Colombian Caribbean environments 2017-2018

Factor	ID	Yield	PC1	PC2	PC3
GEN	1	944.0	-0.1133	-0.3685	-0.2489
GEN	10	1362.1	0.4584	-0.1055	-0.1115
GEN	2	1045.6	0.0531	0.1077	-0.1317
GEN	3	1050.1	-0.2422	0.1296	0.3453
GEN	4	1031.3	-0.4607	0.2078	0.2451
GEN	5	963.2	-0.5175	-0.0113	0.3236
GEN	6	1536.9	1.0000	0.2477	0.3186
GEN	7	1064.8	-0.1562	0.6498	-0.5463
GEN	8	1159.6	-0.1429	-0.3026	-0.0512
GEN	9	1190.0	0.1214	-0.5547	-0.1430
Environment	LE8A	844.0	0.4857	0.4767	0.2326
Environment	MA7B	1404.8	-0.1934	0.4343	-0.2642
Environment	MO7B	961.6	-0.4690	-0.1942	0.2778
Environment	PN7B	681.1	-0.3844	-0.0254	-0.4579
Environment	PN8A	801.3	-0.2064	0.2969	-0.1838
Environment	SA7B	2015.6	-0.4369	-0.6144	0.1511
Environment	SA8A	858.3	0.2045	0.1024	0.5357
Environment	VN8A	1511.4	1.0000	-0.4764	-0.2913

ID: Identification of genotype and environment. GEN (genotype): 1. LC-02916; 2. LC-002-016; 3. LC-036-016; 4. LC-009-016; 5. LC-021-016; 6. L-019; 7. LC-006-016; 8. LC-005-016; 9. L-014-016; 10. CAUPICOR 50. Environment: LE8A (Leticia 2018A); MA7B (Mahates 2017B); MO7B (Montería 2017B); PN7B (Polonuevo 2017B); PN8A (Polonuevo 2018A); SA7B (Sampué 2017B); SA8A (Sampué 2018A); VN8A (Villanueva 2018A)

Figure 2. Biplot analysis concerning the graphic representation of PC1 and PC2, allusive to the genotype-by-environment interaction for 10 cowpea bean cultivars evaluated in eight environments of the Colombian Caribbean. 1= LC-029-016; 2=LC-002-016; 3=LC-036-016; 4=LC-009-016; 5=LC-021-016; 6=L-019; 7=LC-006-016; 8=LC-005-016; 9=LC-014-016; 10=Caupicor 50; 1=LE8A; 2=MA7B; 3=MO7B; 4=PN7B; 5=PN8A 2018; 6=SA7B; 7=SA8A; 8=VN8A.



CONCLUSIONS

1. Environmental effects were mainly responsible for the variation detected in the study, highlighting PN7B as the least favorable environment and VN8A and SA7B, as the most favorable for cowpea bean production;
2. Genotypes 6 and 10 showed the highest grain yields, but were influenced by the environments, with higher yield in favorable environments;
3. Genotype 2 showed greater stability and adaptability in the studied environments, with a yield below the average.

ACKNOWLEDGEMENT

The authors express their gratitude to Colciencias, the Universidad de Córdoba and the Fundación Promotora del Canal del Dique, for financing the research project on cowpea beans.

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